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ADVANCE STATEMENT OF PROGRESS ON NUTRITIONAL AND DISEASE TRANSMITTING POTENTIAL OF SEWAGE-GROWN ALGAE

California University
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on

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University of California
Institute of Engineering Research
Berkeley, California
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SUMMARY OF RESEARCH SINCE MAY 1, 1963

INTRODUCTION:

This section is a brief summary of work done from May 1, 1963 to March 1, 1964. Some of the work summarized in this section has been completed, and the remaining is being conducted at present. Thus, studies on pond performance are receiving less emphasis, and attention is being concentrated on producing a maximum algal crop. Similarly, the extent of the work on harvesting by centrifugation will be sharply reduced, while studies on the use of flocculation will receive the major effort. The investigation on DDT and other insecticide content of the algae product, of the supernatant remaining after the algae have been removed, and of the influent sewage will be discontinued unless supplementary funds are received, since such research is beyond the scope of the original grant, and hence sufficient funds are not available for further pursuit. The studies on pesticides reported herein were necessarily preliminary in nature, and were largely done for us by the California State Department of Health and by the Department of Toxicology at the University of Davis. The nutritional and disease transmission studies will be continued--especially those concerned with the influence of harvesting by coagulation on these two phases.

POND PERFORMANCE:

The operation of the pond during the past year was directed towards the production of a high algal concentration and yield rather than to the attainment of a high degree of efficiency of waste treatment. However, despite the de-emphasis on waste treatment, the centrifuged effluent characteristics continued on the excellent level

noted in the period of research covered in the accompanying report. The BOD of the centrifuged morning effluent ranged from 28 mg/liter during January to 7 mg/liter in May; and that of the afternoon centrifuge effluent, from 25 mg/liter in January to 4 mg/liter in May. Per cent removal of the incoming morning BOD ranged from 50 per cent in January to 84 per cent in April; and that of the incoming afternoon BOD, from 85 per cent in January to 96 per cent in May.

The magnesium content of the centrifuged pond effluent varied from 11 to 38 mg/liter and averaged 19 mg/liter; which represents an average removal of 45 per cent of the incoming magnesium. The calcium content of the centrifuged effluent ranged from 11 to as high as 38 mg/liter, and averaged 26 mg/liter. Percentage removal was strongly influenced by climate, as was shown by the fact that on an average, only 25 per cent of the incoming calcium was removed during the October to March period, whereas from March to August, removal averaged 49 per cent. The phosphate content of centrifuged pond effluent ranged from 4 to 10 mg/liter, and represented a removal of 50 to 79 per cent of the incoming phosphate. At this point, it should be emphasized that these are characteristics of centrifuged effluent. On the basis of results obtained in previous research, values for effluent concentrations of magnesium, calcium, and phosphate would be much lower in the former category and higher in the latter when flocculation is used in harvesting algae.

The ammonium-nitrogen content of the centrifuged effluent and percentage removal of incoming ammonium-nitrogen was influenced by climatological conditions. Thus, the ammonium-nitrogen concentration

of the effluent averaged 1.3 mg/liter and the per cent removal, 92 per cent in the April-May period, whereas they were 9 mg/liter and 50 per cent respectively in the January-February period. A similar trend was noted with respect to total nitrogen. Thus, during the April-May period, the concentration of the total nitrogen in the centrifuged effluent was 5 mg/liter and the per cent removal, 79 per cent; whereas, in the January-February period the values were respectively, 19 mg/liter and 44 per cent. The concentration of sulfate in the centrifuged effluent averaged 73 mg/liter; and percentage removal, 18 per cent.

The outstanding result of the treatment given the pond was that the concentration of algae remained above 130 mg/liter throughout the winter, and at no time were rotifers able to make any inroads. As during the first year, Scenedesmus quadricauda was the predominant organism, while Chlorella was next in abundance.

HARVESTING AND COLIFORM SURVIVAL:

During the first part of the year, initial concentration and dewatering were accomplished in one operation with the use of the DeLaval basket type centrifuge intended for use as the secondary centrifuge in the completed installation. Drying was done by spreading dewatered slurry in a thin layer on aluminum sheets exposed to the sun. Despite the inadequacy of the equipment, approximately 200 lbs of algae were produced during this period. The installation of an 8-inch Buflovac drum dryer brought the total production of air and drum dried algae prior to the completion of the harvesting installation to about 500 lbs.

Results reported in this section on harvesting and coliform

survival were obtained with the use of the following set-up: A Dorr-Oliver Merco B-20 continuous centrifuge (see accompanying report for full description) was used for the initial concentration of the pond effluent; a 12-in by 24-in DeLaval solid bowl centrifuge was used for the secondary concentration; and the final drying was done on a 36-inch wide Buflovac steam heated drum dryer. At times, drying was completed by air drying.

At a throughput rate of 92 gal/min, the removal of algal cells averaged 89.6 per cent, while the concentration of the underflow (concentrate) averaged 13 gm/liter (dry weight) of slurry. The dewatered algal slurry had an average moisture content of 84.7 per cent after being discharged from the DeLaval solid bowl centrifuge. The efficiency of cell removal by the DeLaval centrifuge ranged from 39 to 82.5 per cent, depending upon the frequency of the unloading operation. As the cake built up in the machine, the greater was the tendency to unload some of the accumulated algae. After exposure to the drum dryer, the algal product had an average moisture content of 28.9 per cent. The relatively high average value reflects those values obtained when the steam pressure in the drums was lower than that of the required level. Thus, at 60 psi, the moisture content was as high as 40 per cent; whereas at 80 psi, it ranged from 13 to 18 per cent.

The membrane filter technique was used to ascertain the bacterial count, both coliform and total. In conjunction with these standard counts, numerous bacterial types, actinomycetes, fungi imperfecti, and yeasts were isolated. Attempts to isolate typhoid and paratyphoid organisms were unsuccessful. Sewage coliform counts were found to be generally stable and predictable, ranging from 10^5

to 10^6/ml for coliforms and from 10^8 to 10^9/ml for total counts. Pond coliforms varied from 10^1 to 10^5/ml. At an average feed rate of 332 liters/min to the Merco B-20, and an underflow (concentrate) rate of 49 liters/min, the per cent of the incoming coliforms concentrated in the algae paste averaged (average of 5 runs of 8 hours each) 19 per cent. The coliform count of pond liquid coming into the machine during the five runs averaged 150/ml, and that of the underflow (concentrate) 200/ml. The remaining 81 per cent apparently was discharged in the centrifuge effluent.

The coliform and enterococci count of the algae product was zero when drying was completed on the drum. The total bacterial count of the dried algae varied from 6×10^3 to 3.2/gram of dried algae. Undoubtedly the major fraction of the total count was due to recontamination as a result of handling, since the total counts were made of samples obtained during routine processing.

ANIMAL FEEDING EXPERIMENTS WITH ALGAE:

Animal feeding tests conducted thus far were concerned with the palatability and digestibility of the algal product.

Palatability Trial:

Four mature wethers were used to determine the palatability of air dried algae. The rations were: Ration A, 30 per cent algae, 30 per cent barley, and 40 per cent hay; Ration B, 60 per cent algae and 40 per cent hay; Ration C, 60 per cent barley and 40 per cent hay; and Ration D, 30 per cent barley, 30 per cent cottonseed meal, and 40 per cent hay. The hay was 50 per cent ground alfalfa and 50 per cent ground oat hay. A 4×4 latin square design with two-week experimental periods was employed. The average daily intakes are shown in Table I.

TABLE I

Average Daily Intake

Intake	Ration A	B	C	D
lb/day (total ration)	2.63	1.95	2.81	3.60
lb/day (algae)	0.79	1.17	-	-

The data indicate that sheep prefer barley and cottonseed meal to algae. However, when 30 per cent algae replaced 30 per cent barley, intake was not greatly reduced.

Digestibility of Algae by Sheep:

Four mature wethers were used in a 4 x 4 latin square design experiment to determine the digestibility of algae. Two different samples of air dried algae and a sample of drum dried algae were compared. The rations were: Ration A, 60 per cent air dried algae I and 40 per cent hay; Ration B, 60 per cent air dried algae II and 40 per cent hay; Ration C, 60 per cent drum dried algae and 40 per cent hay; and Ration D, 100 per cent hay. The hay was 50 per cent alfalfa and 50 per cent oat hay. In the preliminary period the sheep sorted out the algae and ate the hay, therefore all rations were pelleted to prevent sorting. All animals were maintained in positive nitrogen balance. A 7-day collection period was used. The analyses of the feeds are shown in Table II and the digestion coefficients are summarized in Table III.

TABLE II

Composition of Feeds

	Dry Matter	Nitrogen	Ether Extract	Crude Fiber	Cellulose	Ash
Air dried algae I	91.98	7.65	1.97	7.83	2.94	18.55
Air dried algae II	91.66	7.36	2.05	8.78	2.89	20.68
Drum dried algae	91.91	7.11	3.97	8.40	3.43	24.21
Oat hay	91.06	1.16	1.20	31.44	30.42	8.67
Alfalfa hay	91.42	3.07	1.47	30.84	29.64	9.64

TABLE III

Summary of Digestion Coefficients

Ration	Dry matter %	Crude Protein %	Ether extract %	Cellulose %	Organic matter %
A. 60% air dried algae and 40% hay[1]	58.3	71.4	27.8	51.9	63.4
B. 60% air dried algae II and 40% hay	57.4	71.8	15.0	50.3	62.6
C. 60% drum dried algae and 40% hay	56.1	74.0	61.0	38.3	62.4
D. 100% hay	63.2	72.7	33.0	60.6	64.6

[1]Hay composed of 50 per cent alfalfa hay and 50 per cent oat hay.

The dry matter digestibility of the algae-containing rations was lower than that for hay. However, there was little difference in the digestibility of organic matter, therefore, the observed difference in dry matter digestibility must be due to the high

mineral content of algae. The crude protein digestion coefficients were similar for all rations. Drum drying appeared to increase ether extract digestibility. This apparent increase may be due to the difficulties in obtaining accurate values for ether extract. Drum drying appeared to decrease cellulose digestibility. The cellulose digestibility of hay was greater than that of algae.

Swine Trial:

Four rations were fed with levels of 0, 2½, 5, and 10 per cent of air dried algae, respectively. The percentage of crude protein in these rations varied from 15 to 16 per cent on an air-dry basis. Algae were added at the expense of the protein components of the rations, and the rations were as follows:

Ground barley	79½	79½	79½	80½
Alfalfa meal	5	5	5	5
Meat and bone scraps	6	5½	4	4
Soybean oil meal	4½	3½	3	-
Cottonseed oil meal	4½	3½	3	-
Algae	-	2½	5	10
Salt	½	½	½	½

Three groups of 4 littermate gilts were divided at random among the 4 rations. The pigs weighed about 84 lb initially; 900 lb of each ration was mixed and fed individually to the hogs over a 46-day trial.

One gilt on the 5 per cent algae level scoured badly and was removed from the trial and sacrificed. Another on the 2½ per cent algae ration scoured some and performed badly. These two animals were

not included in the calculations, since there was no reason to believe their outcome was influenced by the treatment. The results are as follows:

Percentage algae	0	2½	5	10
Average daily gain, lbs.	1.70	1.62	1.60	1.66
Average daily feed, lbs.	6.54	6.08	6.15	6.48
Feed per unit gain	3.86	3.85	3.85	3.90

There was no obvious differences among the groups and the pelleted feed was readily consumed.

NUTRITIVE CHARACTERISTICS:

Complete chemical analyses of 11 weekly samples of algae were made to obtain information as to the uniformity of the product. The average ash content was 14.2 per cent (dry weight, σ = 1.6%); nitrogen, 8.5 per cent (σ = 0.3%); ether extract, 6.8 per cent (σ = 1.2); crude fiber, 4.7 per cent (σ = 0.6); lignin, 4.2 per cent (σ = 0.6); phosphorus, 2.2 per cent (σ = 0.5); calcium, 1.9 per cent (σ = 0.6); cellulose, 3.5 per cent (σ = 0.6); and silica, 1.7 per cent (σ = 0.4). The variation in ash undoubtedly was a function of air drying. On windy days, dust and dirt may have blown into the drying algae.

Analyses by gas-liquid chromatography of algae samples in their native and in their processed states for their lipid components demonstrated the presence of a number of unsaturated fatty acids similar to those in higher plants as well as a new unidentified component. Phosphatidyl ethanolamine, serine, choline, and

galactolipid components were detected. The lipid extracts are undergoing continued investigation to determine their relative abundance and fatty-acid composition.

Studies of the constitution of the cell wall with purified enzymes, although not as definitive as may be desired, indicate the presence of significant amounts of beta-galactosides at the surface of the cell wall. Judging from the literature to date, this has not been previously indicated. This finding tends to confirm observations made in x-ray diffraction studies by others that the constitution of the cell wall of these algae is a mixed co-polymer. These results would indicate that a predigestion by a suitable mixture of enzymes will be necessary for increasing the utilization of algal nutrients by non-ruminants. No activity was obtained with the use of pectin esterases or chitinases.

The rat feeding tests conducted by Dr. B.B. Cook were repeated, except that drum dried algae were used in place of the infra-red or air dried algae used by Dr. Cook. Results obtained in the present research showed algae to be significantly more digestible than the slow-heat dried (infra-red) and air dried algae used by Dr. Cook. With the drum dried algae, growth was obtained which was equivalent to 93 per cent of that obtained with casein. These results compare favorably with those obtained with the use of the much more expensive process of lyophilization. Drum dried algae contain sufficient essential fatty acids when constituting 20 per cent of the protein intake of the total diet to support adequate growth. This indicates that the lipid components are much more well preserved in rapid heat drying than in air drying. These results are analogous with those noted by researchers working with alfalfa. In processing alfalfa, flash drying results in

significant improvement in carotene preservation over that obtained with air drying. Carotene is extremely labile to oxidative degradation.

DDT AND OTHER PESTICIDE CONTENT:

First Run: Fifty grams of algae were extracted by Soxhlet extraction with 110 ml hexane (normal). The extract was passed through a Davidow column (9 ml fuming and 9 mg concentrated sulfuric acid on 30 gms of celite). A gas chromatographic analysis (electron capture detector) was then made. Using the extract on a QF 1 column and on a Dow 11 column failed to show the presence of any pesticide that would not be destroyed by the clean-up procedure at the following limits of sensitivity:

Substance	Limit of Sensitivity (mg/liter)
o, p-DDT	1
p, p'DDT	1
p, p'DDD	0.5
Lindane (BHC)	0.05
Heptachlor	0.1
Heptachlor epoxide	0.1
p, p'-DDE	0.2
Methoxychlor	5
Toxaphene	10
Chlordane	1

In an analysis with paper chromatography, interference precluded identification, but chlorinated organic compounds appeared to be present.

Gas chromatographic procedures failed to give any better results when a Florisil cleanup was used.

Run 2: In other experiments, direct hexane extracts gave many peaks by electron capture gas chromatography which did correspond to known column retention time for lindane and came close to the retention time of aldrin, dieldrin, DDE, p, p'-DDT and p, p'-DDD on a QF 1 column. However, when re-run on a Dow 11 column, the retention time did not correspond to these substances. These findings must be interpreted in terms of the limiting sensitivities given in the first run, which in turn are primarily dictated by the presence of interfering peaks in the vicinity of the retention times for the chemical entities listed. On direct extraction, aldrin and dieldrin would not be destroyed by the cleanup (Passage through the Davidown column outlined previously, would result in the destruction of these compounds); these were not found at a limit of sensitivity of 0.2 mg/liter.

Run 3: Ten grams of algae were extracted with 20 ml of hexane and sent to the Department of Agriculture for analysis. Technicians at the Department of Agriculture passed the extract through a Florisil column before making an analysis by gas chromatography on a Dow 11 column, using a Dohrmann micro electrocoulometric detector (sensitive to chlorides formed by burning the organic chloride effluent of the gas chromatographic column). The Department of Agriculture reported 2.0 mg/liter of DDT, which was confirmed by paper chromatography. This is consistent with the findings made in the local laboratory, allowing for sensitivities, since the department of Agriculture report on DDT would be a sum of the isomers and metabolites, and would

not be subject to electron capture interferences unless they happened also to have carried organic halides (an unlikely circumstances).

Run 4: Run 4 was done in much the same manner as was run 3, except that the sample was extracted by the Department of Agriculture technicians with the use of petroleum ether instead of hexane. They are at a loss to explain their failure to clean up this sample.

Discussion: The results indicate the need for an intensive investigation of the problem of pesticide concentration by algae. Among the important problems brought to light by the work done thus far is the one of determining whether or not algae contain an interfering substance that might result in false readings. The present experiments furnish reason for believing that this may indeed be possible. This can be solved by using algae grown in pure culture, or at least not grown in a sewage medium. It will also involve making determinations of the amount of pesticide in sewage, and in general, all of these studies outlined in the supplementary proposal.